

REMARKS

I. Amendments to the Specification

The amendments to the specification on pages 6, 12, 18, 26 and 27 are typographical in nature, are made to place the application in better form for allowance, and do not add prohibited new matter.

The amendment to page 23 clarifies the nomenclature used in the table. Support for this change is presented in the specification on page 10, lines 20 and 25 and on page 11, line 4, and would be commonly understood by the skilled artisan. No prohibited new matter is added.

The specification has also been amended to incorporate reference to SEQ ID Nos. A request to use the CRF sequence listing from parent application 09/852,000, as well as a paper copy of the sequence listing, are attached hereto.

II. Amendments to the Claims

The amendments to the claims are submitted to redraft the claims in independent form, and to place the claims in essentially the same format as they were at the close of prosecution of parent application 09/852,000. In addition, claims 1-8, 10, 11 and 13 have been canceled. Newly submitted claims 15 and 16 correspond to claims 19 and 20 in parent application 09/852,000. Claim 17 is newly submitted and finds support at page 23, line 18 of the specification.

No prohibited new matter is added by these amendments or claims.

III. Office Action Dated July 9, 2003 in Parent Application 09/852,000

In the Office Action mailed July 9, 2003, in parent application 09/852,000, the Examiner had rejected various claims, including claims 8, 11 and 13, as containing subject matter which was not described in or enabled by the specification. Specifically, the Examiner had asserted that claims to sequences comprising mutations other than those recited allegedly lacked written description and enablement. Without necessarily agreeing with the rejections, Applicants have canceled claims 8, 11 and 13 in favor of claims directed to amino acid sequences that differ from SEQ ID No. 1 by only the recited mutations. Therefore, the written description and enablement rejections set forth in parent application 09/852,000 have no relevance to the pending claims.

Claims 9, 12 and 14 were rejected in parent application 09/852,000 under 35 U.S.C. §112, second paragraph, for alleged indefiniteness. According to the Office Action mailed July 9, 2003, amending the claims to recite “DNA encoding a fluorescent protein comprising the amino acid sequence that differs from SEQ ID NO: 1 by only mutations . . .” would obviate this rejection. Applicants have incorporated the Examiner’s suggested language into the amended claims above. Therefore, the §112, second paragraph rejection set forth in parent application 09/852,000 has no relevance to the pending claims. In this regard, Applicants acknowledge with appreciation the indication in the Office Action mailed July 9, 2003, that claims 12 and 14 would be allowable if rewritten to overcome the rejection under §112, second paragraph.

Claims 8 and 9 and dependent method claims were rejected in parent application 09/852,000 under 35 U.S.C. §103(a) as being unpatentable over Siemering *et al.* in view of Thastrup *et al.* Specifically, Siemering *et al.* was cited as disclosing mutations Val163Ala and Ser175Gly, and enhancement of fluorescence intensity by about 4-fold at 37°C as compared to 30°C. Thastrup *et al.* was cited as disclosing that GFP proteins with at least the mutation of Phe64Leu have higher fluorescence at 37°C than 30°C. These references are allegedly combinable to render the claimed DNAs obvious. Applicants respectfully traverse this rejection with regard to the amended claims above.

Applicants respectfully note that the combination of the Siemering reference with the Thastrup reference was made in hindsight with knowledge of Applicants' invention in hand. There is nothing in Siemering that would teach or suggest the protein having each of the mutations encoded by the DNA of claim 9. Likewise, there is nothing in Thastrup that would teach or suggest the protein having each of the mutations encoded by the DNA of claim 9.

Further, as Applicants have noted during prosecution in parent application 09/852,000, according to MPEP 2143.01, in evaluating obviousness, all teachings in the prior art must be considered to the extent that they are in analogous arts. *In re Young*, 927 F.2d 588, 18 USPQ2d 1089 (Fed. Cir. 1991). Applicants respectfully submit that one of ordinary skill in the art would not have been motivated to use the Phe64Leu mutation of Thastrup *et al.* to achieve increased fluorescence in view of the known deficiencies of the Clontech enhanced BFP (EBFP) variant. Indeed, Angres & Green (published by Clontech Laboratories) teaches that excitation of the

EBFP variant comprising the mutation Phe64Leu “turned out to be quite destructive to the chromophore and photobleaching was a considerable problem” (see first full paragraph). This observation was confirmed by the authors of the Ellenberg *et al.* reference, who state that, while the commercially engineered variant EBFP shows good spectral separation from green emitting variants, it is “dim” and “photobleaches extremely rapidly” (see paragraph bridging pages 842-44).

In the Office Action of July 9, 2003, in parent application 09/852,000, the Examiner responded to Applicants’ arguments, asserting that the Angres & Green and Ellenberg *et al.* references were not prior art, and that the Clontech EBFP variant has had commercial success despite its drawbacks (see page 14 of Office Action). Applicants respectfully submit that the Clontech EBFP variant was known in the “prior art” as evidenced by the April 1997 Clontechniques paper concerning the EBFP variant (of record). The Angres & Green and Ellenberg *et al.* references are evidence that when those of skill in the art used this variant, they found photobleaching to be a considerable problem. In view of these exemplary reports, the skilled artisan would not have been motivated to combine the Phe64Leu mutation of the EBFP variant with other mutations.

The Examiner asserts that the Clontech EBFP variant has had commercial success despite its drawbacks, but Applicants fail to see how commercial use alone would motivate the skilled artisan to use the EBFP mutations to design enhanced fluorescent variants, particularly if reports in the literature indicate that the properties of this variant are less than ideal. A lack of adequate

choices of BFP variants offered by other commercial entities could result in the skilled artisan using EBFP despite its drawbacks, which in no way would make it obvious for someone developing a variant with enhanced fluorescence to use the mutations of EBFP given the reports in the literature and the experience of the skilled artisan. In any case, the Examiner has not presented any evidence or analysis of sales relating to the Clontech EBFP variant to support the assertion that this variant has enjoyed commercial success.

Thus, in contrast to the argument set forth in the Office Action, one of ordinary skill in the art would not have been motivated to use the Phe64Leu mutation disclosed by Thrastup *et al.* to achieve improved fluorescence since the EBFP mutant designed by Clontech containing this mutation was reportedly “dim” and exhibited considerable photobleaching. Further, there would have been no reason to believe that the Phe64Leu mutation would lead to improved fluorescence when combined with other mutations, since it did not lead to improved fluorescence when combined with the Ser65Thr, Tyr66His and Tyr145Phe mutations in the EBFP mutant.

In view of the above remarks, Applicants respectfully submit that the rejection under §103(a) as set forth in the Office Action mailed July 9, 2003, in parent application 09/852,000, is not applicable against claim 9 as amended herein.

Finally, claim 16 and dependent method claims were rejected in parent application 09/852,000 under 35 U.S.C. §103(a) as being unpatentable over Tsien *et al.* in view of Siemering *et al.* Without agreeing with the rejection, Applicants respectfully note that the subject matter of claim 16 from parent application 09/852,000 is not included in the pending

claims. Accordingly, the §103(a) rejection based on Tsien *et al.* and Siemering *et al.* is not applicable to the pending claims.

CONCLUSION

For the reasons set forth above, entry of the foregoing amendment and allowance of the application respectfully is requested. If there are any other filing or claim fees due in connection with the filing of this preliminary amendment, please charge the fees to our Deposit Account No. 50-0310. If a fee is required for any extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

The Examiner is invited to telephone the undersigned if a discussion might expedite placing the claims in condition for allowance.

Respectfully submitted,

MORGAN, LEWIS & BOCKIUS LLP

Date: June 10, 2004

By: Bonnie Weiss McLeod
Bonnie Weiss McLeod
Reg. No. 43,255

Customer No. 009629
MORGAN, LEWIS & BOCKIUS LLP
1111 Pennsylvania Avenue, N.W.
Washington, D.C. 20004
202.739.3000 (Telephone)
202.739.3001 (facsimile)